Niclosamide, a drug with many (re)purposes

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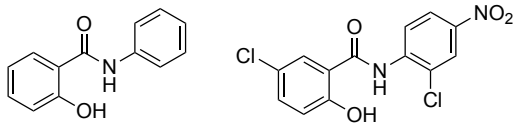
Niclosamide is an anthelmintic drug that has mainly been used for over 50 years to mainly treat tapeworm infections. However, with the increase in drug repurposing initiatives, niclosamide has emerged as a true hit in many screens against various diseases. Indeed, from being an anthelmintic drug, it has now shown potential in treating Parkinson's disease, diabetes, viral and microbial infections as well as various cancers. Such diverse pharmacological activities are a result of niclosamide's ability to uncouple mitochondrial phosphorylation and modulate a selection of signaling pathways, such as Wnt/β-catenin, mTOR and JAK/STAT3, which are implicated in many diseases. In this highlight, we will discuss the plethora of diseases that niclosamide has shown promise in treating.

Following its discovery in the early 1950s, niclosamide (**Figure 1**) was initially used as a molluscicide to kill snails[1] and around a decade later, it was found to be effective against human tapeworm infection.[2] Its use in humans to treat tapeworm infection started in 1982 following FDA-approval and has since been one of the drugs in the World Health Organization's essential list of medicines. Structurally, niclosamide belongs to a large group of lipophilic, weakly-acidic molecules called salicylanilides (**Figure 1**).[3]



Salicylanilides **Niclosamide**

Figure 1. Chemical structures of salicylanilides and niclosamide.

Despite its wide use as an anthelmintic drug, the mechanism of action of niclosamide is yet to be completely understood. Early studies have linked niclosamide's activity to the uncoupling of oxidative phosphorylation.[4] However, over the last decade or so, niclosamide has been shown to act on other targets such as Wnt/β-catenin, mTOR and JAK/STAT3 signaling pathways [reviewed by Chen et al[5]], which inevitably linked niclosamide's therapeutic potential to many diseases that involve these important signaling cascades. The increasing interest over the last decade in niclosamide is reflected in the significant rise in the number of publications that studied this drug (**Figure 2**). Herein, we briefly discuss the numerous diseases niclosamide has shown promise in treating.

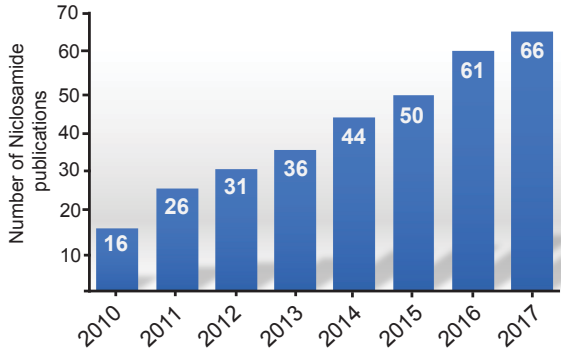


Figure 2. The number of niclosamide papers published since 2010. The data was obtained from PubMed (https://www.ncbi.nlm.nih.gov/pubmed/) using the search term 'niclosamide' and fixing the dates from 1st January to 31st December of each indicated year.

Pharmacological activities of niclosamide

1. Parkinson's disease (PD)

As mutations that impair the catalytic activity of the mitochondrial serine/threonine protein kinase PINK1 (PTEN-induced kinase 1) were found to cause early onset PD[6] through neuronal apoptosis, it became apparent that small molecules that activate PINK1 would have neuroprotective effects and thus have the potential to treat PD.[7] Equipped with the fact that PINK1 is activated by mitochondrial depolarization, Barini et al.[8] investigated whether niclosamide[9], which is known to cause mitochondrial depolarization, could activate PINK1. The results showed that niclosamide was a potent activator of PINK1 in cells. The study showed that niclosamide was not a direct activator of PINK1 as no activation was observed when recombinant PINK1 was incubated with PINK1 in vitro. However, it was shown that niclosamide caused mitochondrial depolarization, a phenomenon that was reversible following the removal of niclosamide from the cell media.[8] Impressively, niclosamide was also shown to activate PINK1 in cultured neurons. Together, this study highlighted the PINK1-mediated neuroprotective promise niclosamide may hold. Further in vivo studies in PD disease models are needed to establish the ability of niclosamide to treat this disease.

2. Type 2 Diabetes Miletus (T2DM)

Niclosamide ethanolamine, the salt form of niclosamide, which exhibits improved aqueous solubility was shown to improve the symptoms of T2DM in mice.[10] Indeed, niclosamide treatment of mice fed on high fat diet led to improved metabolism, increased lipid oxidation and high energy expenditure. Additionally, in db/db mice wherein diabetes is induced as a result of mutations causing deficiency in leptin receptor activity, niclosamide ethanolamine was shown to be effective in improving glycemic control, treating hyperglycemia and slowing down the progression of the disease. In a subsequent study, niclosamide piperazine, which is another

salt form of niclosamide, was also found to have comparable efficacy in treating T2DM.[11] It has been suggested that niclosamide's anti-diabetes therapeutic potential is due to the inhibition of the glucagon signaling PKA pathway.[12]

3. Bacterial infections

Niclosamide has also shown antimicrobial activities against various bacterial infections such as Methicillin-resistant *Staphylococcus aureus* (MRSA)[13] [14], Tuberculosis (TB)[15], and Anthrax[16]. In a drug repurposing study using a library of FDA approved drugs, niclosamide was found to specifically inhibit the growth of the Gram-positive bacteria and to display a strong in vivo and in vitro activity against MRSA (MIC = 0.125 µg/ml).[13] Such finding showed that niclosamide was as effective as vancomycin, the current drug of choice for treating MRSA.[13] Subsequently, niclosamide was investigated for its potential as an antimicrobial surface coating against device associated and hospital acquired infections.[14] Highly versatile niclosamide-based antimicrobial coatings were developed and these were shown to clear existing infections and prevent biofilm formation at very low concentrations.[14]

Niclosamide was also shown to have significant anti-tuberculosis (TB) activity (MIC = 0.5-1 µg/ml).[15] Interestingly, niclosamide exerted pharmacological activity against stationary phase tubercle bacilli and multidrug resistant tuberculosis. However, it was noted that there was potential toxicity to mammalian cells when niclosamide was used at MIC and hence topical use of niclosamide against surface-located tuberculosis may hold better therapeutic promise.

Furthermore, niclosamide was identified to be able to significantly protect cells from an anthrax lethal toxin as well as from lethal factor Pseudomonas exotoxin fusion protein and diphtheria toxin at low micromolar concentrations.[16] This activity was linked to obstructing the process of anthrax toxin internalization and directly linked to endosome acidification.[16]

4. Viral infections

Niclosamide has been suggested for the potential use as a broad-spectrum antiviral to target host pathways used by viruses for infection unlike the current antiviral strategies which are directed against a specific viral target.[17] This broad antiviral activity of niclosamide has been linked to its ability to neutralize the endosomal pH and as a result disrupting the pH-dependent membrane fusion required for the virus entry.

To date, several studies have indicated the potential use of niclosamide to treat Coronaviruses (SARS-CoV and chikungunya virus)[18], Zika[19] and Ebola viruses.[20]Niclosamide was discovered to be able to inhibit the coronavirus, SARS-CoV replication at a low micromolar concentrations. Viral antigen synthesis was totally abolished at niclosamide concentration as low as 1.56 µM.[18] The exact mode of action is not clear, but niclosamide was found not to interfere with the virion's attachment and entry into cells as it was still active even when added 3 hours after viral infection of cells. Some *in silico* studies suggested the potential binding of niclosamide to the SARS-CoV main proteinase.[19] However, only a modest activity of niclosamide against this proteinase was reported (IC_{50} = 40 µM).[20] Moreover, niclosamide exhibited antiviral activity against chikungunya virus (CHIKV) using CHIKV 26S mediated insect cell fusion screening assay and was able to inhibit virus entry and cell-to-cell transmission of CHIKV infection[20]. In light of the recent outbreak of Zika and Ebola infection diseases, there was focus on the drug repurposing screening of FDA approved drugs for the rapid identification of potential therapeutic agent to meet the urgent medical need to treat these infections. Using such approach, niclosamide was identified as an inhibitor of the Zika virus.[21] Further validation assays confirmed the ability of niclosamide to significantly reduce Zika RNA levels and suppress the production of infectious Zika particles at sub-micromolar concentrations.[21] Subsequently, niclosamide emerged as a positive hit from another drug repurposing screen aimed at identifying clinically used agents that may have activity against the Ebola virus (EC_{50} = 1.5 µM).[22]

5. Cancer

By far, the disease that has received the most attention in the repurposing of niclosamide is cancer. To date, niclosamide has been shown to exhibit anticancer activity against colon,[23] breast,[24] prostate,[25] glioblastoma,[26] osteosarcoma,[27] ovarian,[28] leukemia,[29] adrenocortical carcinoma,[30] lung[31] and oral[32] cancers.[5]

These observed anticancer activities have been linked to niclosamide's ability to damage tumor cell mitochondria, induce apoptosis, inhibit tumor cell proliferation and inhibit various aberrant tumor signaling pathways including Wnt/β-catenin, mTORC1, STAT3, nuclear factor-κB (NF-κB) and Notch pathway (reviewed in detail by Moskaleva et al[33]). In addition to this broad spectrum anticancer activity, niclosamide was also shown to inhibit cancer stem cells,[34] which are considered an attractive and promising anticancer therapeutic target since accumulating evidence has established their crucial role in cancer initiation, progression, establishment, recurrence and drug resistance.[35] Beyond its use as a single agent, niclosamide has been studied in combination with conventional chemotherapeutic agents. For instance, combination of niclosamide with cisplatin led to the inhibition of cisplatin-resistant triple negative breast cancer[36] and exhibited synergistic effect in cisplatin-resistant lung cancer cells[37]. Enhanced sensitivity of chronic myeloid leukemia cells to dasatinib[38] and that of cervical cancer cells to paclitaxel[39] was also achieved when these drugs were used in combination with niclosamide. Synergistic effects with erlotinib in colon cancer[40], head and neck cancer[41] and in erlotinib-resistant non-small lung cancer cells[31] have also been described. While the combination of niclosamide with bicalutamide led to overcoming enzalutamide and bicalutamide-resistant prostate cancer,[42] the combination with abiraterone was found to be effective in treating advanced castration resistant prostate cancer[43]. Furthermore, the use of niclosamide in conjunction with radiotherapy resulted in significant radio-sensitizing effects in triple-negative breast cancer cells[44] and overcame radio-resistance in lung cancer [45].

Considerations for the use of niclosamide as a therapeutic

Along with the interest in repurposing niclosamide for these various diseases, it must be noted that niclosamide's ability to influence many signaling pathways may work to its disadvantage as it would lead to diverse side effects. Also, the favorable safety profile of niclosamide in treating humans with tapeworm infection could be due to the fact that the site of action in this case is the gut and hence the drug is not absorbed to modulate various signaling cascades and cause side effects. With the newly discovered diseases where niclosamide may hold a therapeutic effect, systemic delivery maybe required and hence the safety profile of niclosamide in this case is still largely unknown. Current and future in vivo studies on niclosamide will provide a clearer

picture of its efficacy and toxicity, which will determine the success of these repurposing initiatives.

Conclusion

Niclosamide has shown great promise in treating many diseases through the uncoupling of oxidative phosphorylation and the manipulation of various cell signaling cascades. With this comes some safety concerns may arise following its systemic exposure. Insights into these safety issues will be attained in the near future from the various ongoing niclosamide clinical investigations.

Statement. As this is a short Highlight of niclosamide's pharmacological activities, it was impossible to include all of the studies published on niclosamide in this area. The studies discussed in this work are just a representation of the myriad of niclosamide's therapeutic activities.

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Keywords: Niclosamide • Screening • Repurposing • Disease • Cancer

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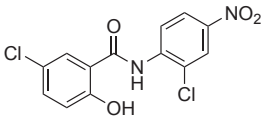

Niclosamide

One drug, potential to treat many diseases:

- Parkinson's disease
- Diabetes
- Bacterial infections
- Viral infections
- Cancer

Many recent drug repurposing studies have identified the anthelmintic drug niclosamide as a potential treatment of numerous diseases. Although the mechanism of action of niclosamide in many cases is not fully understood, its in vitro and in vivo therapeutic effects have resurrected this old drug and generated some excitement about its potential repurposing for various diseases that are currently difficult to treat.